Karyomorphology of Five South Indian Species of *Smilax* Linn.

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Accepted November 12, 1987

Smilax Linn. (Smilacaceae) is a relatively large genus of dioecious plants distributed in the tropical and temperate regions. Concerning the systematic relationships of the Smilacaceae, of which *Smilax* is the type genus, there is great deal of controversy among the different systems of classification. Although information about chromosome morphology in higher plants is known to be of considerable evolutionary and taxonomic significance (Stebbins 1971, Love and Love 1975), karyomorphology of the genus *Smilax* is little known. Karyotype details of four diploid species such as *S. aspera*, *S. bracteata*, *S. wightii* and *S. zeylanica*, and of three polyploid cytotypes of *S. ovalifolia* from South India are reported here.

Materials and methods

Materials of species reported here were collected from various high and low altitude regions in South India such as Vattakottai, Bonacaud, Ponmudi, Peermade, Munnar (Kerala State), Kodaikanal and Ootacamund (Tamil Nadu). Somatic chromosomes were studied from root tips fixed in Carnoy's fluid, and squashed in acetocarmine, and karyotype data taken based on average measurements of chromosomes of 10 well-spread metaphase plates. For evaluation of chromosome morphology, the terminology proposed by Leven *et al.* (1964) and for categorisation of karyotype asymmetry, the scheme proposed by Stebbins (1958) were followed. The TF% was calculated according to Huziwar (1962). For constructing karyotype formula, the chromosome lengths of taxa reported here were classified under five size groups such as A=12–9 μm ; B=9–6 μm ; C=6–4 μm ; D=4–2 μm and E=2 > μm .

Observations

Smilax aspera Linn.

Root tip cells of both male and female plants showed 32 chromosomes which ranged from 7.00–2.30 μm in length. The karyotype consisted of 1 pair of m-type, 7 sm-type, 7 st-type and 1 of t-type chromosomes (Fig. 1). The karyotype was fairly asymmetrical (3B; TF%=22.88). Two pairs of chromosomes showed satellits on their short arm (Nos. 7 and 11). Recognisable size heteromorphism was noticed between the members of one of the SAT-pairs (No. 7) in the male plants.

S. bracteata Presl

Root tip cells of male and female plants showed 32 chromosome which ranged from 5.33–1.73 μm in length. The karyotype consisted of 1 pair of m-type, 7 sm-type and 8 of st-type chromosomes (Fig. 2), and it belonged to 3B class of asymmetry with TF%=25.96. One of the medium sized pairs was satellited (No. 7).

S. wightii A.D.C.

Thirtytwo chromosomes were observed in the root tip cells of both male and female plants,

and they ranged from 5.32–1.67 μm in length. The karyotype consisted of 1 pair of m-type, 8 sm-type and 7 of st-type chromosomes (Fig. 3). The longest chromosome pair showed a secondary constriction at the middle of the long arm, and chromosome number 12 was satellited. The karyotype was fairly asymmetrical (3B; TF%=25.42).

S. zeylanica Linn.

Root tip cells of plants of both the sexes showed 32 chromosomes which ranged from 9.66–3.10 μm in length. The karyotype consisted of 1 pair of m-type, 6 sm-type, 8 st-type and 1 of t-type chromosomes (Fig. 4). One of the medium sized pairs (No. 8) had a globular micro-satellite on the short arm. The karyotype was fairly asymmetrical (3B; TF%=22.16).

S. ovalifolia Roxb

This species occurs in this region in three polyploid ocytotypes ($2n=64, 96$ and 128), and they exhibited recognisable degree of difference in some of the vegetative characters (Vijayavalli and Mathew 1986).

Table 1. Summary of karyotype data in five species of *Smilax*

Taxa	2n	Ploid	TCL	ACL	Karyotype formula	Asymmetry	TF%
<i>S. aspera</i>	32	2x	126.60	3.96	K(2n)=32: 2Bst+2Bt+4Csm+6Cst+2Dm+10Dsm+6Dst	2B	22.88
<i>S. bracteata</i>	32	2x	105.58	3.31	K(2n)=32: 2Csm+6Cst+12Dsm+10Dst+2Em	3B	25.96
<i>S. wightii</i>	32	2x	91.88	2.87	K(2n)=32: 2Cst+2Ct+14Dsm+8Dst+2Em+2Esm+2Est	3B	25.42
<i>S. zeylanica</i>	32	2x	168.34	5.26	K(2n)=32: 2Ast+8Bst+6Csm+4Cst+2Dm+4Dsm+4Dst+2Dt	3B	22.16
<i>S. ovalifolia</i>							
Cytotype I	64	4x	230.30	3.60	K(2n)=64: 4Csm+18Cst+14Dm+18Dsm+8Dst+2Esm	3B	27.67
Cytotype II	96	6x	257.50	2.68	K(2n)=96: 8Cst+2Ct+8Dm+22Dsm+24Dst+2Dt+4Em+18Esm+8Et	3B	25.74
Cytotype III	128	8x	449.56	3.51	K(2n)=128: 6Bst+2Bt+16Csm+16Cst+2Ct+8Dm+38Dsm+38Dst+2Dt	3B	24.99

TCL=Total chromosome length. ACL=Average chromosome length.

Cytotype I

The plants of this type, collected from Ponmudi and nearby forest ranges, showed 62 chromosomes in root tip cells (Fig. 8). The somatic chromosomes could be broadly classified into three size groups such as 8 pairs of large sized (6.00–5.13 μm), 8 medium sized (4.66–3.16 μm) and 16 of smaller ones (2.99–1.83 μm). The karyotype consisted of 7 pairs of m-type, 12 sm-type, and 13 of st-type chromosomes (Fig. 5). The karyotype belonged to 3B category with TF%=27.67.

Cytotype II

The plants of this, collected from Munnar had 96 chromosomes in root tip cells of both the sexes, and they ranged from 4.84–1.67 μm in length (Fig. 9). The karyotype consisted of 6 pairs of m-type, 20 sm-type, 20 st-type and 2 of t-type chromosomes (Fig. 6). The karyotype belonged to 3B category with TF%=25.74.

Cytotype III

Materials of this were collected from high elevation forest ranges in Munnar, Kodaikanal

1. *Smilax aspera* (male) $2n = 32$

2. *Smilax bracteata* $2n = 32$

3. *Smilax wightii* $2n = 32$

4. *Smilax zeylanica* $2n = 32$

5. *Smilax ovalifolia* $2n = 64$

Figs. 1-5.

and Ootacamund. Root tip cells showed 128 chromosomes which ranged in size from 6.67–2.00 μm (Fig. 10). The karyotype consisted of 4 pairs of m-type 27 sm-type, 30 st-type and 3 of t-type chromosomes (Fig. 7), and it belonged to 3B type of asymmetry with $\text{TF}\% = 24.99$.

6. *Smilax ovalifolia* $2n = 96$

7. *Smilax ovalifolia* $2n = 128$

Figs. 6–7.

Discussion

Chromosome data on *Smilax* show that the genus is multibasic with $x=13$, 14, 15 and 16, of which $x=16$ is the most frequent. All the South Indian species are based on $x=16$. It has been suggested that $x=16$ could be the earlier evolved basic number in the genus from which the other lower ones evolved by descending aneuploidy, and that the $x=16$ itself could

be a secondary condition evolved from an ancestral lower one by polyploidy (Vijayavalli and Mathew 1987). Karyomorphological data on the species reported here show that their karyotypes are predominated by st-and t-type chromosomes (Table 1), and they all have low values of TF% (27.67–22.16). Chromosomes are also small sized with ACL ranging from 5.26–

Figs. 8–10. Root tip chromosomes in three polyploid cytotypes of *Smilax ovalifolia*. All figures $\times 1200$. 8, cytotypic I, $2n=64$. 9, cytotypic II, $2n=96$. 10, cytotypic III, $2n=128$.

2.68 μm . This together with appreciable intrakaryotypic size difference of chromosomes make their karyotypes strikingly asymmetrical (3B). The karyotypes of all the diploid species are apparently similar in gross karyomorphology as they all have the same karyotype asymmetry factor (3B) and comparable TF% values. However, in finer details of some of the individual

chromosomes, they show recognisable degree of difference (Fig. 11). *Smilax zeylanica* has the highest chromatin content (TCL: 168.34 μm and ACL: 5.26 μm) as against the lowest in *S. wightii* (TCL: 91.88 μm and ACL: 2.87 μm). As regards centromere positions, the chromosome No. 1 is the most variable one, which in *S. aspera* is highly acrocentric ($r=9.48$). A few other chromosomes (Nos. 5, 6, 7, 8, 10, 11 and 15) show varying degrees of difference in centromeric position. Of the four diploid species, *S. zeylanica* has the maximum karyotype asymmetry, and *S. bracteata* the least. In *S. aspera* chromosome No. 7 was found to be heteromorphic in male plants, and based on this, an XY (male) and XX (female) type of sex chromosome complex has been assumed for this species (Vijayavalli and Mathew, 1987).

Bentham and Hooker (1883) and several other earlier as well as a few of the recent taxonomists (Emberger 1960, Melchior *et al.* 1964, Hamann 1961 etc.) have included the genus *Smilax* in their Liliaceae under the tribe Smilacaceae. But, Hutchinson (1959, 1973) has treated this under a separate family (Smilacaceae) in his Liliales. His consideration for erecting the family was purely exomorphic distinction of its members, all characterised by woody habit, leaves with reticulate venation, unisexual flowers, confluent anther loculi etc. Karyomorphological data on *Smilax* show that its members possess small sized (ACL: 3.96–2.68 μm) and

Fig. 11. Arm ratio of chromosomes of four-diploid species of *Smilax*.

mostly subtelocentric chromosomes. Their karyotypes are fairly asymmetrical (3B with low TF% values) as a result of their chromosomes having had undergone large scale structural repatterning. These attributes make the genus distinct from the great bulk of the Liliaceous genera which possess large sized chromosomes and much less specialised karyotypes. A survey of chromosome numbers of the various tribes and genera of the conventional Liliaceae show that, with respect to chromosome number also, the genus *Smilax*, (with predominantly $x=16$) is significantly different from the other genera of Bentham and Hooker's Liliaceae. Phytochemical information has revealed that *Smilax* has a fatty acid pattern different from that of the Liliaceae in having less linoleic, more palmitic and small amounts of tetrasanoic acid (Dahlgren 1983). Thus the chromosome features of *Smilax*, considered together with the uniqueness of its members in respect of exomorphic and phytochemical characters, lend support to the removal of the genus from the Liliaceous assemblage, and justify its elevation to a separate family status.

Although several of the recent taxonomists are also agreed on the creation of a separate family around *Smilax*, there is considerable discord concerning its systematic relationships and affinities. Novak (1954) has placed Smilacaceae under his suborder Lilineae and Kimura (1956) in his Liliales, but keeping Agavaceae also in the assemblage. Huber (1969, 1977) and Dahl-

gren and Clifford (1982), who favoured the erection of a separate family (Smilacaceae) however, have placed it in their Asparagales. Takhtajan, although earlier had placed Smilacaceae under the Liliales (1959, 1969), has subsequently (1980) created a new order (Smilacales) around the family. Cronquist (1968, 1981), who also placed Smilacaceae in the Liliales along with Liliaceae, Amaryllidaceae and Agavaceae, has however, pointed out that the genus *Smilax* differs from the great bulk of the Liliaceae in having vessels distributed throughout the plant as well as in the more obvious character of the habit. He has also considered that the genus in several respects has parallelism to members of the Dioscoreaceae. Dyel (1955) has accommodated Smilacaceae in his Dioscoreales together with Dioscoreaceae, while Takhtajan (1980) kept these two families in his order Smilacales. It may be noted that members of the Smilacaceae and Dioscoreaceae share many features in common such as small-sized chromosomes, dioecious nature, climbing habit, reticulate venation etc. In respect of chemical characters also they show appreciable similarity due to rich occurrence of steroidal saponins (Dahlgren, 1985). On account of these similarities, the proposed relationship and affinity between Smilacaceae and Dioscoreaceae appear to be stronger, and their treatment together in the same order seems sensible.

Summary

Detailed karyomorphology of four diploid species of *Smilax* such as *S. aspera* ($2n=32$), *S. bracteata* ($2n=32$), *S. wightii* ($2n=32$), *S. zeylanica* ($2n=32$) and of three polyploid cytotypes of *S. ovalifolia* ($2n=64, 96, 128$) from South India has been studied.

All the species are based on $x=16$. Their chromosomes are small sized, and karyotypes strikingly asymmetrical (3B and with low TF% values). The diploid species are similar in gross karyomorphology; but in finer details recognisable difference is evident in a few chromosomes. In *S. aspera*, one chromosome (No. 7) is heteromorphic in male plants.

Systematic relationships of the genus *Smilax* is viewed in the light of chromosome data. The chromosome features lend support to the removal of the genus from the Liliaceous assemblage, and to the creation of a separate family (Smilacaceae) around it. The proposed affinity between Smilacaceae and Dioscoreaceae is favoured.

Acknowledgements

The authors are thankful to Prof. C. A. Ninan, Head of the Department of Botany for helpful suggestions, encouragements and facilities. One of us (BV) is grateful to the University Grants Commission, the Department of Collegiate Education and the Sree Narayana Trust for financial support and study leave benefits.

References

- Bentham, G. and Hooker, J. D. 1883. *Genera Plantarum*. Vol. II. Reeve, London.
- Cronquist, A. 1968. *The Evolution and Classification of Flowering Plants*. Thomas Nelson (Printers) Ltd. London.
- 1981. *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.
- Dahlgren, R. M. T. 1983. The importance of modern serological research for angiosperm classification. In Jenson, U. Fairbrothers, D. E. (ed.) *Proteins and nucleic acids in plant systematics*. Springer Verlag, Berlin, Heidelberg, New York.
- and Clifford, H. T. 1982. *The Monocotyledons, A comparative study*. Academic Press, London.
- , — and Yeo, P. F. 1985. *The families of the Monocotyledons, Structure, Evolution and Taxonomy*. Springer Verlag, Berlin, Heidelberg, New York.
- Dyel, M. 1955. The evolution of plants and the taxonomy of Monocotyledons. *Acta. Mus. Nat. Pragae*. XI

- B. No. 6. Bot. No. 3: 1-143.
- Emberger, L. 1960. "Traite de Botanique" Vol. II. Les vegetaux vasculaires (M. Chaudefaud and Emberger ed.) Nesson *et cie*. Paris.
- Hamann, U. 1961. Merkmalsbestand und Verwandtschaftsbeziehungen der "Ferinasse". *Welldenowia* 2: 639-768.
- Huber, H. 1969. Samenmerkmal und Verwandtschaftsverhältnisse der Liliflorae. *Mitteil Bot. Staatssumml. Munchen* 8: 219-538.
- 1977. The treatment of the Monocotyledons in an evolutionary system of classification. *Plant Syst. Evol. Suppl.* 1: 285-298.
- Hutchinson, J. 1959. The Families of Flowering Plants. Vol. II. Monocotyledons. Oxford, Clarendon Press.
- 1973. The Families of Flowering Plants. Vol. I. Clarendon Press, London.
- Huziwara, Y. 1962. The karyotype analysis in some genera of Compositae. IX. The chromosomes of some European species of *Aster*. *Bot. Mag.* 75: 143-150.
- Kimura, Y. 1956. System *et* Phylogeneic des Monocotyledons. *Notules Syst.* 15: 137-159.
- Levan, A. K., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
- Love, A. and Love, D. 1975. Plant Chromosomes. J. Cramer, Inder, A. R. Gartner Verlag. Commanditgesellschaft.
- Melchior, H. (ed.) 1964. "A Engler's syllabus der Pflanzen Familien" 12. Aufl. Band. II. Angiosperm Borntrager. Berlin.
- Novak, F. A. 1954. System Angiosperm *Preslia* 26: 337-364.
- Stebbins, G. L. 1958. Longevity, habitat and release of genetic variability in higher plants. *Cold Spring Harb. Symp. Quart. Biol.* 23: 365-378.
- Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Addison-Wesley Publishing Co., California.
- Takhtajan, A. 1959. "Die Evolution der Angiospermen". G. Fisher. Jena.
- 1969. "Flowering Plants, Origin and Dispersal" Oliver and Loyd. Edinburgh.
- Takhtajan, A. 1980. Outline of the Classification of Flowering Plants. *Bot. Rev.* 46: 225-359.
- Vijayavalli, B. and Mathew, P. M. 1986. New report of polyploid cytotypes in *Smilax ovalifolia*. *Curr. Sci.* 55: 666.
- and — 1987. Cytology of five species of *Smilax* from South India. *The Nucleus* 30: 57-60.
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